Ethiopian International Journal of Multidisciplinary Research

pISSN:2349-5707 Volume:12,Issue 10,October-2025 eISSN:2349-5715

UDC: 631.527:578.2:582.736.3

EVALUATING MOLECULAR IDENTIFICATION TECHNIQUES FOR PATHOGENIC FUNGI ON PISTACHIOS: A COMPREHENSIVE LITERATURE REVIEW ON PRIMER MARKERS AND METHODOLOGICAL GUIDELINES

Odina Nazarova Jumadullaevna, PhD in agriculture
odinanazarova.on@gmail.com

Research institute of Forestry, Uzbekistan

Abstract. This article reviews molecular identification methods applied to pathogenic fungi affecting pistachio cultivation. In particular, the use of PCR and primer markers is analyzed, emphasizing their advantages in terms of accuracy, rapidity, and efficiency compared to traditional morphological methods. The importance of selecting appropriate primers for differentiating fungal species is highlighted, as well as the application of microsatellite markers, PCR-RFLP, and phylogenetic analyses. The article demonstrates the opportunities for integrating modern molecular diagnostics into agricultural practice, with the ultimate goal of safeguarding pistachio yield and quality.

Keywords: Pistachio, phytopathogenic fungi, molecular identification, PCR, primer markers, PCR-RFLP, microsatellites, phylogenetics, sustainable production.

Introduction. The careful identification of pathogenic mushrooms is essential to mitigate the impact of diseases on pistachio crops, which are economically significant in the agricultural sector. The growing incidence and variety of fungal pathogens addressed by pistachio farmers require advanced identification methods that can help in a reliable way in disease management strategies. Molecular diagnostics, particularly the polymerase chain reaction techniques (PCR) have gained traction as essential tools in this regard. According to Nooralden and Mohammed [13], the implementation of the PCR not only improves specificity and speed compared to traditional identification methods, such as morphological and cultural approaches, but also increases the sensitivity of detection, allowing the confirmation of pathogens even at low inoculo levels.

Materials and Methods.

Literature Review Approach. This study was conducted as a comprehensive literature review focusing on molecular techniques applied to the identification of pathogenic fungi in pistachio cultivation. Peer-reviewed articles, technical reports, and recent case studies were analyzed to synthesize methodological advances and their practical applications in plant pathology and sustainable agriculture.

Polymerase Chain Reaction (PCR) and Primer Design. Polymerase chain reaction (PCR) served as the primary diagnostic tool examined. Studies reviewed demonstrate that PCR significantly improves sensitivity, specificity, and speed in pathogen detection compared to traditional morphological and cultural approaches. Particular emphasis was placed on the selection of species-specific primer sequences, which ensure accurate amplification and allow differentiation of closely related fungal taxa. Nested PCR assays were also evaluated for their role in detecting low-level infections in pistachio orchards.

Microsatellite (SSR) Markers. Microsatellite markers (simple sequence repeats, SSRs) were identified as an essential methodology for genetic characterization of both pathogens and pistachio genotypes. These markers facilitate the detection of genetic variability among fungal populations and enable insights into host–pathogen interactions, contributing to resistance breeding strategies (fig.1)

PCR-RFLP Analysis. Restriction fragment length polymorphism (PCR-RFLP) was reviewed as a robust approach for species differentiation, particularly within *Aspergillus* spp., which are common contaminants in pistachio production (fig. 2).

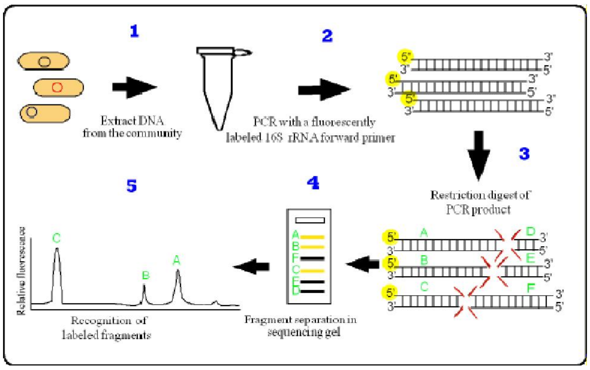

Figure 1. Depicts the workflow of SSR marker analysis.
Source: fessto.weebly.com – Genetic marker workflows

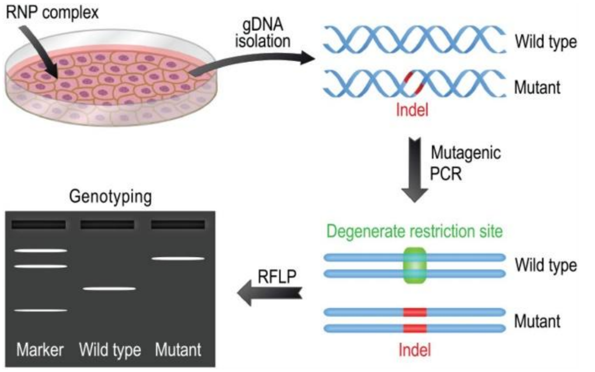

Figure 2. PCR-RFLP workflow.
Source: bioRxiv (A PCR-RFLP method for the detection of CRISPR-induced indels)

This method enhances the discriminatory power between closely related species and provides rapid, cost-effective results relevant for food safety and quality control.

Molecular Phylogenetics. Molecular phylogenetic analysis was assessed for its utility in clarifying taxonomic relationships within pathogenic genera such as *Alternaria* and *Botryosphaeria*. By using ribosomal DNA and gene-specific markers, phylogenetic approaches support a more precise classification of pathogens and improve understanding of their evolutionary dynamics.

Next-Generation Sequencing (NGS) and Multiplex PCR. High-throughput sequencing and multiplex PCR technologies were included in the review as modern techniques enabling complete profiling of fungal communities in pistachio ecosystems. These methods provide insights into species diversity, ecological interactions, and environmental factors influencing pathogen prevalence, thereby offering a holistic perspective on pistachio pathobiomes.

Morphological Identification (Comparative Framework). Although morphological and cultural techniques were acknowledged as traditional diagnostic tools, the reviewed literature consistently highlighted their limitations. These approaches were compared against molecular methods, underscoring the greater accuracy, reproducibility, and reliability of DNA-based diagnostics.

Results. The diversity of fungal pathogenic agents that affect pistachios is considerable, with studies that reveal that species such as *Botryospharia Dothidea*, *Fusarium Solani, Aspergillus Flavus* are among the most prevalent and economic harmful [14]. These mushrooms represent significant risks to producing pistachios, in regions such as California, where environmental conditions favor their growth and proliferation. The pathogen of these mushrooms is not only linked to their ability to cause a visible decay, but also to their ability to produce mycotoxins, which can affect both agricultural productivity and food safety.
Molecular techniques, such as PCR, are evolving to meet the specific needs of the pistachio industry. First, primer markers play a crucial role in the effectiveness of these methodologies.

The selection of appropriate primer sequences for PCR is vital to ensure accurate amplification of the Target fungin DNA. The research conducted by Ma and Michailides [10] clarifies the development of specific primers by species that allow the rapid differentiation of pathogenic mushrooms that affect pistachios. Their results highlight the importance of primer design based on genetic variability between fungal taxa, which helps not only in accurate identification, but also in the detection of strictly related species that may not be distinguishable using traditional techniques.

Recent progress in molecular identification also includes high -performance sequencing technologies and multiplex PCRs, which allow complete profiling of microbial communities in pistachio orchards. Trouneillas et al. [20] underline the usefulness of these modern approaches, demonstrating how they can discover the complex interactions within the phyllosphere and the fungal populations of the radical area. Using these sophisticated techniques, researchers can better understand the ecology of pathogenic mushrooms and their prevalence in different microhabitats associated with the cultivation of pistachio.

The selection of methodologies for the molecular identification of pathogenic mushrooms must be considered several factors. These include the ability to detect a wide range of fungal species, the specificity of the primers used, the speed of the diagnostic process and the effectiveness in terms of costs for practical agricultural applications. In addition, researchers are encouraged to standardize the protocols between studies to ensure consistency and comparability of the results. These guidelines are crucial to establish a picture for the integration of molecular diagnostics in the routine management practices of pistachio cultivation. Continuous research and innovation in molecular identification methodologies will be essential to effectively deal with the evolving challenges posed by fungal pathogens in the production of pistachios. The revision of literature will focus on the variety of primer markers used in fungal identification, evaluating their meaning in improving the detection methodologies for pathogenic mushrooms that affect pistachios. A fundamental progress in this domain is the implementation of microsatellite markers, as indicated by Ahmad et al. [1] (fig.3). Their research highlighted the usefulness of the microsatellite loci in the genetic characterization of different varieties of pistachios, facilitating the differentiation of fungal pathogenic agents based on the susceptibility of the guest. This technique not only helps to identify the strains of pathogenic agents but also contributes to a wider understanding of the interactions of the guest-patrogen, meaning the profound relevance of microsatelli markers in the plant pathology.

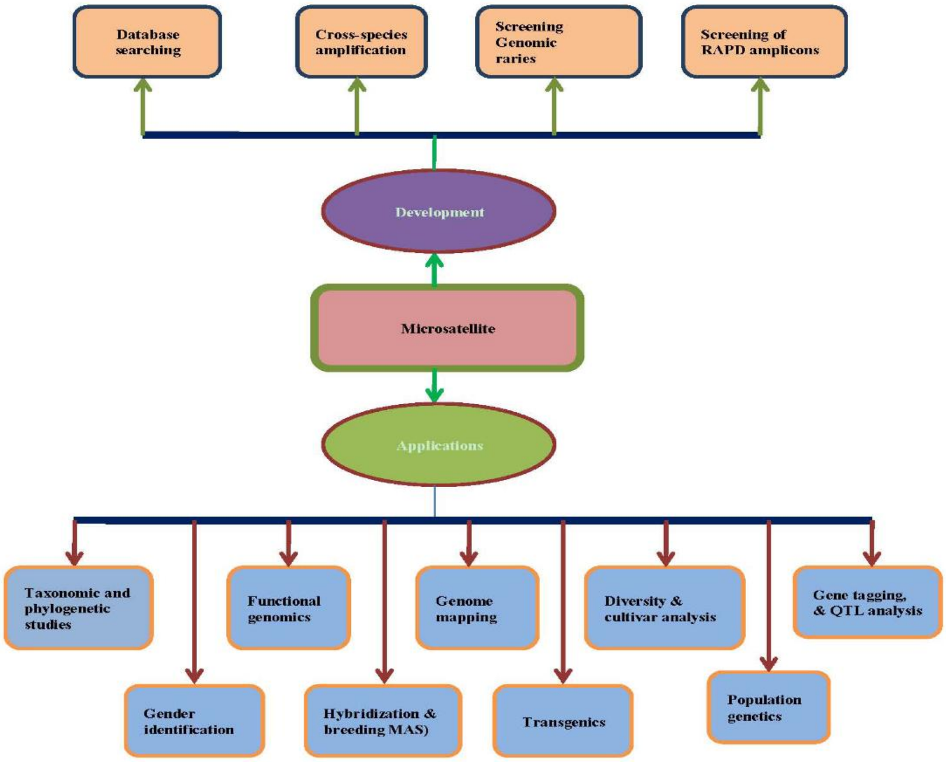

Figure 3. Development and applications of microsatellite markers
Source: IJMS review on microsatellite markers

In the reign of molecular diagnostics, the analysis of the length of the length of the fragment of chain reaction of the polymerase chain polymerase) emerged as a robust method for the differentiation of various species of Aspergillus. Alaayadi et al. [2] evaluated this technique and reported its effectiveness in the rapid identification of Aspergillus spp., Contaminants of the common pistachios. Their results have shown that PCR-RFLP not only provides quick results but also improves the discriminatory power between strictly related fungal species. The study underlined the potential for this methodology of improving quality control in agricultural practices, about food safety and post-cacculted losses.

In addition, Ozkilinc and Sevinc [15] contributed significantly to the understanding of the phylogenetic relations between the alternating pathogens associated with pistachios. Their analysis used molecular phylogenetics to clarify the taxonomic diversity of alternating species, which are known to cause leaf points and other diseases in various guest plants, including pistachios. The identification of specific genetic markers linked to these pathogenic species is crucial for the development of targeted management strategies. This study acts as a precious resource for the selection of appropriate markers who not only see in accurate identification but also facilitate understanding of pathogenesis at the molecular level.

Further insights on the genetic variability between the fungal species that affect pistachios can be found in the research conducted by Kilinç et al. [9] and Kafkas [8]. Kilinç et al. Focused on genetic markers associated with fungal populations that infect pistachios, revealing a significant diversity that can influence the dynamics of the disease. On the other hand, Kafkas [8] provided a complete review of the molecular methods applied to fungal species, underlining their variability and implications for the management of crop health. Both studies validate the need to select highly specific and sensitive primer markers capable of producing accurate genetic information relevant for the assessments of pathogenicity.

These collective results illustrate a growing corpus of literature that shows the critical roles of various primer markers in the molecular identification of pathogenic mushrooms in pistachios. The integration of techniques such as microsatellite analysis, PCR-RFLP and phylogenetic studies emerges as essential to refine diagnostic tools, thus improving the precision of fungal identification. This revision of literature will further explore the methodologies and experimental configurations used in these studies to identify optimal primer markers, contributing to the general objective of improving molecular detection methods to encourage sustainable pistachio production systems. In the context of the evaluation of molecular identification techniques for pathogenic fungi that affect pistachios, it is essential to select appropriate methodologies. The discourse surrounding morphological identification techniques Molecular has been widely analyzed in recent literature. Kadkhoda-Hematabadi et al. [7] provided a critical comparison of these methodologies, illustrating that while morphological identification can offer initial information, it often lacks the accuracy required to precisely classify species within the complex fungal communities. This research underlines the inherent limitations of depending solely on phenotypic characteristics, especially given the high level of morphological plasticity exhibited by several fungal pathogens.

On the contrary, molecular techniques have emerged as indispensable tools to achieve a more refined understanding of fungal diversity. Alaei et al. [3] highlighted the genetic diversity of isolated pathogenic fungi of pistachio orchards, demonstrating that molecular markers such as ribosomal DNA and other methodologies based on sequences not only confirm the identity of the species, but also reveal phylogenetic relationships that morphological methods cannot properly capture. This information is crucial when the implications of fungal diversity are considered in the health and management strategies of plants.

When designing methodologies to study pathogenic fungi, integral guidelines are essential. Mainly, researchers should consider the genetic characterization of the objective pathogens and the specificity of the markers of primers used in the polymerase chain reaction tests (PCR). For example, Palavauzis et al. [17] presented a systematic protocol for the selection of primers, emphasizing the importance of whether the primers are specific to particular or capable of recovering various sequences. This nuanced approach facilitates a more precise identification of critical pathogens, improving the reliability of findings in epidemiological studies.

In addition, the practical applications of molecular techniques are exemplified in the work of Gusella et al. [6], who used next -generation sequencing combined with environmental DNA sampling to identify populations of fungi in pistachio orchards. His research not only supported the specific detection of species often overlooked by traditional methods but also provided information on environmental factors that influence the distribution and abundance of fungi. These applications highlight the need to incorporate advanced molecular tools in standard methodologies to study plant pathobiomas.

The implications for future research in the field of pistachio fungal pathobiomas are significant. As indicated by Belair et al. [4], ongoing challenges include the great diversity of fungi associated with pistachios, as well as the environmental variations that can influence the prevalence of pathogens and virulence. To address these challenges, there is an urgent need for a synergistic approach that integrates molecular techniques with ecological and agronomic considerations. By advancing in the understanding of interactions within fungal communities and their reception plant, researchers can facilitate the development of more effective management strategies.

In summary, the intersection of morphological and molecular methodologies provides an integral framework for the identification and study of pathogenic fungi in pistachios. The collective ideas obtained from literature establish a solid basis for future research, advocating the systematic adoption of advanced molecular identification techniques in plant pathology. This systematic approach will not only improve the precision and depth of the investigation but will also contribute to the best agricultural practices and the management of diseases in pistachio cultivation.

Citations:

1. Ahmad, R., Ferguson, L., & Southwick, S. M. (2003). Identification of pistachio (Pistacia vera L.) nuts with microsatellite markers. Journal of the American Society for Horticultural Science, 128(6), 898-903.
2. Alaayadi, A., Ali, S., Başbuğa, S., Atik, O., & Can, C. (2020). PCR-RFLP analyses of Aspergillus spp. isolated from pistachio in Turkey. The Journal of Turkish Phytopathology, 49(2), 25-33.
3. Alaei, H., Mirkhani, F., Mohammadi, A., & Haghdel, M. (2024). Genetic diversity of Trichoderma spp. isolated from alkaline pistachio soils using morphological and molecular characteristics. Journal of Advances in Plant Protection, 1(2), 83-96.
4. Belair, M., Pensec, F., Jany, J. L., Le Floch, G., & Picot, A. (2023). Profiling walnut fungal pathobiome associated with walnut dieback using community-targeted DNA metabarcoding. Plants, 12(12), 2383.
5. Crespo, M., Lawrence, D. P., Nouri, M. T., Doll, D. A., & Trouillas, F. P. (2019). Characterization of Fusarium and Neocosmospora species associated with crown rot and stem canker of pistachio rootstocks in California. Plant Disease, 103(8), 1931-1939.
6. Gusella, G., Lawrence, D. P., Aiello, D., Luo, Y., Polizzi, G., & Michailides, T. J. (2022). Etiology of Botryosphaeria panicle and shoot blight of pistachio (Pistacia vera) caused by Botryosphaeriaceae in Italy. Plant disease, 106(4), 1192-1202.
7. Kadkhoda-Hematabadi, S., Mohammadi, H., & Sohrabi, M. (2023). Morphological and molecular identification of plant pathogenic fungi associated with necrotic wood tissues of pomegranate trees in Iran. Journal of Plant Pathology, 105(2), 465-479.
8. Kafkas, S. (2018). Advances in breeding of pistachio. Burleigh Dodds Science Publishing.
9. Kilinç, B., Güldür, M., & Dikilitaş, M. (2022). Determination of infestation rate, morphological and genetic characterization of Neoscytalidium novaehollandiae in pistachio (Pistacia vera L.) trees in Şanlıurfa province.
10. Ma, Z., & Michailides, T. J. (2002). A PCR-based technique for identification of Fusicoccum sp. from pistachio and various other hosts in California. Plant disease, 86(5), 515-520.
11. Ma, Z., Luo, Y., & Michailides, T. J. (2003). Nested PCR assays for detection of Monilinia fructicola in stone fruit orchards and Botryosphaeria dothidea from pistachios in California. Journal of Phytopathology, 151(6), 312-322.
12. Nezami, E., & Gallego, P. P. (2023). History, phylogeny, biodiversity, and new computer-based tools for efficient micropropagation and conservation of pistachio (Pistacia spp.) germplasm. Plants, 12(2), 323.
13. Nooralden, Z. N., & Mohammed, H. A. (2022). Isolation And Identification Of Fungi Associated With Walnuts, Almonds, Hazelnuts, Cashews, Pistachios And Peanuts Using Molecular Diagnostics Technique (PCR) In The Local Markets Of Kirkuk Governorate/Iraq. Journal of Pharmaceutical Negative Results, 13.
14. Nouri, M. T., Lawrence, D. P., Holland, L. A., Doll, D. A., Kallsen, C. E., Culumber, C. M., & Trouillas, F. P. (2019). Identification and pathogenicity of fungal species associated with canker diseases of pistachio in California. Plant disease, 103(9), 2397-2411.
15. Ozkilinc, H., & Sevinc, U. (2018). Molecular phylogenetic species in Alternaria pathogens infecting pistachio and wild relatives. 3 Biotech, 8(5), 250.